Comparative epidemiology of *E. coli* resistance to third-generation cephalosporins in diseased food-producing animals

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ABSTRACT

Categorized by WHO as critically important antibiotics, third-generation cephalosporins (3GCs) are one of the latest therapeutic alternatives to fight severe infectious diseases in humans. Some antibiotics belonging to this class are prescribed to treat food-producing animals in specific pathological contexts. Preserving the effectiveness of 3GCs requires characterization and careful monitoring of 3GCs resistance and the identification and implementation of measures that can limit this antimicrobial resistance (AMR). Here, we characterized the 3GCs resistance in *Escherichia coli* isolated from diseased animals. Using data collected from broilers, hens, calves, piglets, sows, turkeys and ducks between 2006 and 2016 by the French surveillance network of AMR in pathogenic bacteria of animal origin (called RESAPATH), we investigated the dynamics of resistance to 3GCs. Our non-linear analysis applied to time series showed that the evolution of *E. coli* resistance to 3GCs is specific to each animal category. From 2006 to 2010, resistance to 3GCs increased for most animal categories. We observed peaks of high-level of resistance for hens (21.5% in 2010) and broilers (26.7% in 2011), whereas trends stayed below 10% for the other animal categories throughout the study period. Resistance later decreased and, since 2014, 3GCs resistance has dropped below 10% for all animal categories. The parallel between trends and measures to limit AMR over the period shed lights on the impact of practices changes, public policies (EcoAntibio Plan) and sector-led initiatives (moratorium in swine sector). Finally, they highlight the usefulness and importance of AMR surveillance networks in animal health, such as RESAPATH.

1. Introduction

The last-generation cephalosporins (i.e. third-generation (3GCs) and fourth-generation cephalosporins (4GCs)) are one of the last therapeutic alternatives to fight severe infectious diseases in humans (WHO, 2017). Considered as critically important antibiotics (CIA) (Collignon et al., 2009; WHO, 2017), these antibiotics have been classified of highest priority for human medicine. In France, last-generation cephalosporins are used in veterinary medicine primarily to fight infections caused by *Escherichia coli*, a major pathogen in animal health. Four 3GCs and 4GCs antibiotics (cefovecin, cefoperazone, cefquinome and ceftiofur) are licensed and sold in France for animal use. These antibiotics are approved for parenteral administration in calves, beef cattle,

pigs, cats, dogs and horses, for parenteral and intramammary administration in dairy cattle, and they have no label for poultry (Anses-ANMV, 2017a) but *in ovo* injections of 3GCs were reported before 2014 (Anses, 2014).

Resistance to 3GCs and 4GCs is a major public health concern. Around the world, attention mainly focuses on livestock animals due to their potential role in the global transfer of resistant bacteria and resistance genes to humans though the food chain or by contamination of the environment (Hale et al., 2012; Geenen et al., 2013; Woolhouse et al., 2015). In addition, bacteria in farm animals are widely considered as the reservoir for antibiotic resistance genes (Hu et al., 2016). Preserving the effectiveness of 3GCs and 4GCs, requires characterization and careful monitoring of the dynamics of bacterial resistance to

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these antibiotics and identification and implementation of measures that can curb this resistance. In this context, studying resistance to last-generations cephalosporins relies strongly on efficient surveillance and monitoring systems in both humans and animals. Hence, continuous surveillance of resistance at national and international levels is important to quantify the spread of resistance, to guide the design of appropriate policies and antimicrobial stewardship, and to assess the efficacy of sector-led initiatives or measures already implemented (WHO, 2014).

E. coli, a member of the *Enterobacteriaceae* family, is both a common commensal organism of the mammalian intestinal tract and one of the most frequently isolated pathogens in food-producing animals. *E. coli* is also considered to be an excellent sentinel of antimicrobial resistance (AMR) for a wide range of animal species (De Graef et al., 2004; Aarestrup et al., 2008). *E. coli* can potentially confer resistance to other bacteria through horizontal gene transfer and it is a key source of dissemination of resistance genes (Tenover, 2006). In bovine and swine productions, *E. coli* is one of the most frequent etiological agents of digestive disorders in calves and piglets (DebRoy and Maddox, 2001) and the most frequent bacterial species isolated from sows with urinary tract infections (Moreno et al., 2017). In the avian sector, *E. coli* causes mainly acute fatal septicaemia (Dho-Moulin and Fairbrother, 1999). In animal health, ceftiofur is used as antibiotic resistance marker for 3GCs resistance. Previous studies has shown that the resistance of *E. coli* strains to ceftiofur varies according to the animal species considered (Zhang et al., 2017; Boireau et al., 2018b), but these studies have not examined resistance according to age, production type nor pathology. However, due to differences in practices (e.g. production system, antibiotic use and control measures), the evolution of 3GCs resistance in *E. coli* may vary over time and between animal categories (animal species and production types). The description of resistance to 3GCs lays the groundwork for understanding the evolution of resistance and for assessing the potential impact of control measures implemented to limit AMR. A risk analysis perspective requires a more detailed characterization of 3GCs resistance levels and trends in animal health.

The aim of this study was to characterize the trends of resistance to 3GCs in *E. coli* isolates from seven food-producing animal categories (broilers, hens, calves, piglets, sows, turkeys and ducks) in specific pathological contexts between 2006 and 2016. The comparison of resistance trends and policy measures or sector-led initiatives to limit AMR over the period provided the opportunity to examine the potential impact of changes in practices.

2. Materials and methods

2.1. Source of data

This retrospective study was performed using data from the French Surveillance Network for AMR in pathogenic bacteria from animals, called RESAPATH. It is coordinated by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Created in 1982 to monitor AMR in cattle, RESAPATH has expanded to include swine and poultry since 2000 and all animal species since 2007. RESAPATH collects data from antimicrobial susceptibility testing (more than 50,000 in 2016) performed by French veterinary laboratories that have joined the surveillance network voluntarily (ANSES, 2017). Initially, antimicrobial susceptibility testing (antibiograms) are requested by veterinarians in a pathological context for diagnostic purposes. Laboratories participating in RESAPATH use standard bacteriological culture techniques and methods for bacterial isolation and identification. Antibiograms are performed using the disk diffusion method, according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM). Inhibition zone diameters are communicated to ANSES and compiled in a database. The RESAPATH laboratories have been participating in annual ring trials since 2006, which contributes to the quality control of the data.

From the RESAPATH database, we extracted data regarding *E. coli* resistance to ceftiofur from 2006 to 2016. We selected the seven food-producing animal species or production type for which *E. coli* was the most frequent bacterial species isolated by the network: broilers, hens, turkeys, ducks, piglets, sows and calves (the RESAPATH did not collect enough data to investigate 3GCs AMR in fish and rabbits over the period). For each livestock animal category, considering antibiotic use and practices depending on the pathological context, we selected the major disease caused by *E. coli*: septicaemia for poultry, digestive disorder for piglets and calves, genitourinary infection for sows. Thus, variables extracted included animal species, production type, clinical disease, sampling date and inhibition zone diameter. In accordance with the approved breakpoints from the veterinary section of the CA-SFM, we categorized the isolates as susceptible ($S \geq 21$ mm), intermediate ($18 \leq I < 21$) or resistant ($R < 18$ mm).

To examine a parallel between the evolution of resistance of *E. coli* to 3GCs over the period and potential changes in antibiotic use, we listed all the events (regulatory measures, action plan to fight AMR, initiatives by sector professionals) potentially related to the control of antimicrobial use and AMR or change in husbandry practices in animal health in France from 2006 to 2016. We questioned French experts in different livestock animal sectors to compile information on changes in practices. From the French Agency for Veterinary Medicinal Products (ANMV), we collected data regarding the Animal Level of Exposure to Antimicrobials (ALEA) indicators for 3GCs, which represent the levels of exposure to 3GCs through oral and parenteral administrations routes by dividing the weight of animals treated with the weight of the population potentially consuming antimicrobials (ANSES-ANMV, 2017b). As off-label uses are not recorded through sales data (ANSES-ANMV, 2017b), no ALEA does not mean no usage. In addition, we consulted French regulations to identify policy and regulatory measures linked to the control of AMR.

2.2. Data analysis

From an epidemiological point of view, the event of particular interest is the non-susceptibility of the pathogen to ceftiofur, indicating that it is no longer a wild-type strain. Intermediate isolates were therefore grouped together with resistant isolates in the non-susceptible population, referred to as resistant in this study. The indicator of AMR was defined for each animal category as the proportion of the number of resistant strains relative to the total number of strains tested.

In time-series analysis, a trend reflects the structural dynamics of a phenomenon and is used to monitor long-term movements (Bourbonnais and Terraza, 2010), in particular in AMR (López-Lozano et al., 2000; Mühlemann et al., 2006; Doernberg et al., 2015; Boireau et al., 2018b). By definition, a trend is the long-term variation and is one of the components of a time series along with seasonality and noise (i.e. the residual component). The dynamics of 3GCs resistant *E. coli* isolates was modelled using generalized additive models (GAM) (Dominici et al., 2002) to capture trends and seasonality. The time step was chosen so as to be able to include at least 25 antibiograms per time step (Barlow, 2011) and to ensure the validity of the model (independent and normally distributed residuals). For broilers, hens, piglets and calves we used a monthly time step, whereas we chose a bimonthly time step for turkeys, ducks, and sows.

The time-series analysis spanned a period running from January 2006 to December 2016, except for ducks, for which the study period was reduced to 2008–2016 (due to the limited number of antibiograms collected by the network before 2008). Using count data, the monthly or bimonthly number of resistant strains was modelled with a quasi-Poisson regression with an offset equal to the log of the total number of strains submitted monthly or bimonthly. AMR trends were modelled for each animal category and analysed using a separate and independent GAM. The models used in this paper included non-parametric smoothing functions of calendar time selected by cross-validation and